

# Albumin improves formation and detection of some specific protein-DNA complexes in the mobility shift assay

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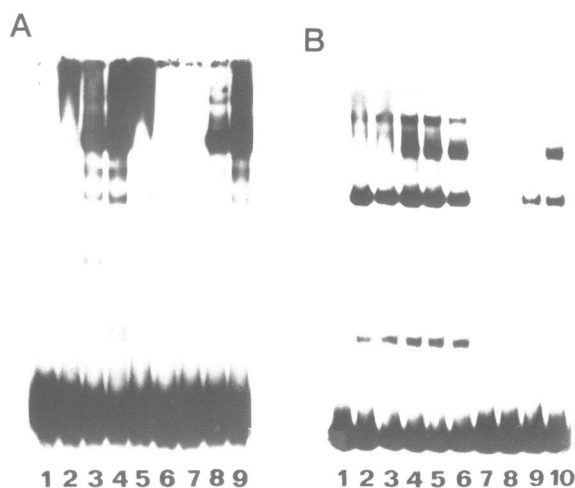
Submitted January 11, 1990

The mobility shift assay (1, 2) is a widely used method for the detection of sequence-specific DNA-binding proteins in crude nuclear extracts. We observed that addition of an indifferent protein (e.g. bovine serum albumin, BSA) to reaction mixtures in the commonly used incubation buffer (25 mM HEPES, pH 7.5; 10 mM KCl; 5 mM MgCl<sub>2</sub>; 1 mM DTT; 1 mM EDTA; 10% glycerol) may promote formation of novel protein-DNA complexes (Fig. 1). These complexes resulted from specific protein-DNA interactions as evidenced by the competition experiments with an excess of unlabelled unrelated DNA fragment (Fig. 1A, lanes 6, 7 and Fig. 1B, lanes 7, 8) and unlabelled homologous DNA fragments (Fig. 1A, lanes 8, 9). Complexes were not formed in the absence of BSA, or in the presence of higher amounts of nuclear extracts, or under prolonged incubation periods (data not shown). Increasing the

poly dI-dC content in the reaction mixture was also without effect (Fig. 1A, lane 5). Similar improvement was obtained with other proteins (concanavalin A, soya bean and lentil lectins). Based on this observation detection of specific DNA complexes with proteins present in extracts in low concentration may be considerably improved or even enabled.

## REFERENCES

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**Figure 1.** Autoradiography of a mobility shift assay experiment. HeLa cell nuclear extract (3) and DNA fragments from the genome of pseudorabies virus (4) were used. Incubation with 1.5  $\mu$ g of nuclear proteins was carried out for 15 min on ice in the final volume of 10  $\mu$ l and in the presence of 1  $\mu$ g poly dI-dC (unless otherwise indicated). **A.** <sup>32</sup>P-end-labelled *Sau*3AI fragment A extending from nucleotide 11710 to 12002 (4). Lane 1, control without nuclear extract; lane 2, no BSA; lanes 3, 4, 6–9, 2 mg/ml BSA; lane 5, 5  $\mu$ g poly dI-dC; lanes 6, 7, 100-fold and 200-fold molar excess of unlabelled fragment A, respectively; lanes 8, 9, 200-fold molar excess of unrelated unlabelled fragment B (nucleotides 4642 to 4830) or C (4830 to 5300), respectively (4). **B.** <sup>32</sup>P-end-labelled fragment B (nucleotides 4642 to 4830) (4). Lane 1, no nuclear proteins and 2 mg/ml BSA. Lanes 2, 7, 9 no BSA; lane 3, 0.25 mg/ml BSA; lane 4, 0.5 mg/ml BSA; lane 5, 1 mg/ml BSA; lanes 6, 10, 2 mg/ml BSA. Lanes 7, 8, 50-fold excess of unlabelled fragment B; lanes 9, 10, nuclear proteins were incubated with double the amount of the labelled fragment B for 15 min, then the binding reaction was divided into two portions and to one of them (lane 10) BSA was added and incubation continued for another 10 min.